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Title: Radiochemical Assessment of Glycogen Synthase Enzyme Activity in Animal Tissue

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length
Number of Steps: 05
Number of Shots: 13

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

What is the scope of your research? What questions are you trying to answer?

1.1. **Bryce Holt:** Our research investigates glycogen metabolism, particularly glycogen synthase, in health and disease, using genetically engineered mouse models.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the most recent developments in your field of research?

1.2. **Bryce Holt:** Research on glycogen storage diseases primarily centers on developing treatments that minimize the buildup of toxic glycogen
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

What significant findings have you established in your field?

1.3. **Bryce Holt:** We demonstrated that our siRNA delivery system effectively reduced glycogen synthesis in a mouse model of glycogen storage disease.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

What research gap are you addressing with your protocol?

1.4. **Bryce Holt:** This protocol describing high-throughput assessment of tissue glycogen synthase enzymatic activity using radioisotopes currently has no published protocol.
   1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.2.1*

What advantage does your protocol offer compared to other techniques?

1.5. **Bryce Holt:** Protocol advantages are direct measurement of enzyme activity and high sensitivity, integral for use on samples with low enzyme activity.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.4.4*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Ball State University

Protocol

2. Radiochemical Glycogen Synthase Activity Reaction

Demonstrator: Bryce Holt

2.1. Begin by taking the prepared murine skeletal muscle homogenate and the [14C]-UDP-glucose *(14-Carbon-U-D-P-Glucose)* reaction mix plate.

2.1.1. WIDE: Talent at the working bench with sample homogenate and reaction mix placed in front of him.

2.2. Using a multichannel pipette, add 25 microliters of the sample supernatant to the designated reaction wells **[1]**. Add 25 microliters of homogenization buffer to the blank and total wells **[2]**. Record the exact time at which the reaction is initiated for each row **[4]**. Ensure that equal volumes are added across all wells **[5]**. Videographer's NOTE: 2.2 was filmed altogether

2.2.1. Talent pipetting 25 microliters of sample supernatant into reaction wells using a multichannel pipette.

2.2.2. Talent adding 25 microliters of homogenization buffer to the blank and total wells. **TXT: Add one row at a time, waiting ≥1 min between rows**

2.2.3. Talent records the time of reaction initiation.

2.2.4. Close-up shot showing even liquid volumes across wells on the reaction plate.

2.3. Allow the reaction to proceed for 10 to 15 minutes while making sure that all rows are run for an identical duration to maintain consistency **[1]**. Videographer's NOTE: 2.3 removed from the steps

2.3.1. Talent keeps the plates aside and starts the timer.

2.4. Afterward, mix the reaction in each well by pipetting up and down several times **[1]**. Using a multichannel pipette, aspirate 55 microliters from each well **[2]** and dispense the liquid onto the corresponding chromatography papers prepared earlier **[3]**. Spot the liquid carefully at the center of each paper **[4]**. Videographer's NOTE: 2.4 & 2.5 were filmed together

2.4.1. Talent pipetting up and down to mix the contents of each well.

2.4.2. Talent aspirating 55 microliters from the wells using a multichannel pipette.

2.4.3. Talent dispensing the aspirated liquid onto chromatography papers aligned in the template.

2.4.4. Close-up shot of the liquid being spotted at the center of the chromatography paper.

2.5. Using forceps, remove the chromatography papers labeled as totals from the clamp after spotting the liquid **[1]**. Place these total papers on aluminum foil to air-dry safely **[2]**. Drop all other chromatography papers into a stirring 66 percent ethanol bath **[3-TXT]**. Videographer's NOTE: 2.4 & 2.5 were filmed together

2.5.1. WIDE: Talent using forceps to remove the total chromatography papers from the clamp.

2.5.2. Talent placing the total papers neatly on aluminum foil for drying.

2.5.3. Talent submerging remaining chromatography papers into a stirring 66 percent ethanol bath. **TXT: Repeat the reaction and spotting for each row of the reaction plate**

Results

3. Results

3.1. Quadriceps glycogen synthase activity in murine skeletal muscle was lower under basal conditions without glucose-6-phosphate **[1]** and significantly higher under maximal conditions with glucose-6-phosphate, consistent with partial phosphorylation of glycogen synthase **[2].**

3.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the two dark gray bars labeled "–GS activity" for female and male samples showing lower values.*

3.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight the two light gray bars labeled "+GS*

- Murine

Pronunciation link: https://www.merriam-webster.com/dictionary/murine
IPA: /ˈmjʊr.aɪn/
Phonetic Spelling: myoor·ine

- Skeletal

Pronunciation link: https://www.merriam-webster.com/dictionary/skeletal
IPA: /ˈskɛl.ə.ɾ̯əl/
Phonetic Spelling: skel·uh·tl

- Homogenate

Pronunciation link: https://www.merriam-webster.com/dictionary/homogenate
IPA: /həˈmɑː.dʒə.neɪt/
Phonetic Spelling: huh·maa·juh·nayt

- [14C]-UDP-glucose

Pronunciation link: No confirmed link found
IPA: /ˌfɔːrˈtiːn ˈkɑːr.bən ˌjuː.diːˈpiː ˈgluː.koʊs/
Phonetic Spelling: for·teen kar·buhn yoo·dee·pee gloo·kohs

- Multichannel

Pronunciation link: https://www.merriam-webster.com/dictionary/multichannel
IPA: /ˌmʌl.tiˈtʃæn.əl/
Phonetic Spelling: mul·tee·chan·uhl

- Microliters

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪ.kroʊˌliː.ɾɚ/
Phonetic Spelling: my·kroh·lee·terz

- Supernatant

Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˌsuː.pɚˈneɪ.tənt/
Phonetic Spelling: soo·per·nay·tuhnt

- Homogenization

Pronunciation link: https://www.merriam-webster.com/dictionary/homogenization
IPA: /həˌmɑː.dʒə.nəˈzeɪ.ʃən/
Phonetic Spelling: huh·maa·juh·nuh·zay·shuhn

- Chromatography

Pronunciation link: https://www.merriam-webster.com/dictionary/chromatography
IPA: /ˌkroʊ.məˈtɑː.grə.fi/
Phonetic Spelling: kroh·muh·taa·gruh·fee

- Ethanol

Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθ.əˌnɔːl/
Phonetic Spelling: eth·uh·nawl

- Quadriceps

Pronunciation link: https://www.merriam-webster.com/dictionary/quadriceps
IPA: /ˈkwɑː.drəˌsɛps/
Phonetic Spelling: kwah·druh·seps

- Glycogen

Pronunciation link: https://www.merriam-webster.com/dictionary/glycogen
IPA: /ˈglaɪ.kə.dʒən/
Phonetic Spelling: gly·kuh·juhn

- Synthase

Pronunciation link: https://www.merriam-webster.com/dictionary/synthase
IPA: /ˈsɪn.θeɪs/
Phonetic Spelling: sin·thays

- Glucose-6-phosphate

Pronunciation link: https://www.merriam-webster.com/dictionary/glucose-6-phosphate
IPA: /ˈgluː.koʊs sɪks ˈfɑːs.feɪt/
Phonetic Spelling: gloo·kohs siks fahs·fayt

- Phosphorylation

Pronunciation link: https://www.merriam-webster.com/dictionary/phosphorylation
IPA: /ˌfɑːs.fə.rəˈleɪ.ʃən/
Phonetic Spelling: fahs·fuh·ruh·lay·shuhn